

wherein each said hybridization condition is selected from the group consisting of:

hybridization in 4XSSC at 65°C, followed by washing in 0.1XSSC at 65°C for an hour,

hybridization in 50% formamide and 4XSSC at 42°C, followed by washing in 0.1XSSC at 65°C for an hour, and

hybridization in 0.5 M Na₂HPO₄ (pH 7.2), 7% SDS, and 1 mM EDTA at 65°C, followed by washing for 15 minutes in 2 x SSC with 0.1% SDS at room temperature, then twice washing for 20 minutes in 0.1 x SSC, 0.1% SDS at 65°C; and

wherein said nucleotide sequence that hybridizes encodes a product that exhibits repression of gene expression activity.

REMARKS

Claims 1-3, 5-9, 11-19 and 31-49 and 51-53 are pending. By this Amendment, claims 50 and 54-56 are canceled and claims 1 and 9 are amended. No new matter is added.

The attached Appendix includes marked-up copies of each rewritten claim (37 C.F.R. §1.121(c)(1)(ii)).

FEBRUARY 20, 2003, AMENDMENT AFTER FINAL REJECTION

The Examiner is requested to enter the Submission filed February 20, 2003. The above amendments and the following comments pertain to amendments to the claims made in the February 20, 2003 submission.

Applicants appreciate the indication in the Advisory Action that the arguments and amendments presented in the February 20, 2003, Amendment After Final Rejection overcome the §112, second paragraph, and the §103(a) rejections.

DRAWINGS

Applicants appreciate the indication in the Advisory Action that the February 20, 2003, proposed drawings corrections have been approved by the Examiner. Applicants concurrently file a Letter to the Official Draftsperson, forwarding the formal drawings to the Patent Office.

III. PREAMBLE OF CLAIM 1

Applicants have amended claim 1 so that the preamble is of a similar scope to the scope of the wherein clause at the end of the claim.

The Advisory Action suggests in the last sentence of the paragraph titled "Continuation of 5", that the only mechanism taught in the present application of regulating gene expression, is that of repression of transcription. This objection was also stated on page 11 (first full paragraph) of the Office Action dated May 8, 2002, where the Office Action suggested that this objection might be overcome by including a statement in the preamble of --repressing transcription of a gene of interest--.

Applicants have again reviewed this suggestion and respectfully traverse this objection and the Office Action's proposed amendment. It is submitted that the present invention discloses the repression of expression of a coding sequence of interest, and this statement has been introduced within the preamble of claim 1. It is noted that the exact mechanism of repression is not fully understood. While the mechanism of regulating gene expression may involve transcriptional repression, other regulatory events may also be involved.

Figures 9, 10 and 17-19 (Examples 2 and 4) indicate that the combination of effector and reporter plasmids results in reduced activity of the product of the reporter plasmid, in this case GUS *enzyme activity*, when compared to the activity of the reporter plasmid in the absence of the effector plasmid. These data demonstrate that gene expression, as demonstrated by reduced *enzyme activity*, is regulated in the presence of the histone

deacetylase. Furthermore, as stated on page 15, lines 17-20, repression of gene expression may involve reducing the levels of mRNA, protein or both. Applicants submit that the scope of presently amended claim 1, which is directed to repressing expression of a coding region of interest, is in keeping with scope of the invention as disclosed in the specification.

Applicants have amended claim 1 so that the preamble is of a similar scope to the scope of the wherein clause at the end of the claim, and removal of this objection is respectfully requested. As a result of the amendment to claim 1, and the above arguments, claim 1 satisfies the requirements of 35 U.S.C. 112, first paragraph.

IV. NUCLEOTIDE FRAGMENTS

Regarding notes 2 and 5 of the Advisory Action, the Advisory Action indicates that nucleotides sequences:

49-267 and 457-534 of SEQ ID NO: 5,
61-855 of SEQ ID NO: 7,
61-655 of SEQ ID NO: 7,
61-276 of SEQ ID NO: 7, and
522-655 of SEQ ID NO: 7,

are not discussed in the specification. Applicants respectfully disagree, as these nucleotides sequences and their associated utility are reasonably inferred from the specification and drawings as initially filed.

Figure 10A of the present application displays several constructs of AtHD2A that were prepared and tested and Figure 10B exhibits the associated activity of these constructs. The numbering indicated in Figure 10A refers to the amino acid sequence. The nucleotides defined in claims 1 and 9 that have been objected to refer to the nucleotide sequence corresponding to the amino acid positions identified in Figure 10A. Figure 2A of the present application indicates the relationship between the amino acid and nucleotide sequence of AtHD2A. The table provided on page 11 of the February 20, 2003 Amendment After Final

Rejection lists the corresponding nucleotide and amino acid sequence numbering for SEQ ID NO:5.

Therefore, the nucleotide positions relating to SEQ ID NO:5 that are listed in claims 1 and 9 of 49-267 and 457-534, refer to amino acids 1-73 and 137-163. As stated in the application, amino acids 1-73 correspond to the catalytic residues of histone deacetylase (see page 28, lines 4-6), and amino acids 137-163 correspond to the acidic domain of histone deacetylase (see page 27, line 28 to page 28, line 4).

As stated on page 27, line 28 to page 28, line 9, page 37, line 30 to page 38, line 8, and page 37, line 22 to page 38, line 8, the residues associated with catalytic activity and the acidic domain are conserved in the histone deacetylases, AtHD2A, AtHD2b and ZmHD2. Based upon the homology between AtHD2A (SEQ ID NO:5) and AtHD2B (SEQ ID NO:7) that can be determined from Figures 3 and 4, it can readily be established that the nucleotide positions identified in claims 1 and 9 associated with SEQ ID NO:7 correspond to similar positions as those identified in SEQ ID NO:5. Figure 2B of the present application indicates the relationship between the amino acid and nucleotide sequence of AtHD2B. The Table included on page 11 of the February 20, 2003 response lists the corresponding nucleotide and amino acid sequence numbering for SEQ ID NO:7.

Therefore, the nucleotide positions relating to SEQ ID NO:7 that are listed in claims 1 and 9 of 61-855, 61-655, 61-276 and 522-655, refer to amino acids: 1-265 and 1-198 (the portion of histone deacetylase including the catalytic domain and acidic region); 1-72 (the catalytic domain), and 154-198 (the acidic region), respectively.

As stated in the application, amino acids 1-73 of AtHD2A correspond to the catalytic residues of histone deacetylase (see page 28, lines 4-6), and amino acids 137-163 correspond to the acidic domain of histone deacetylase (see page 27, line 28 to page 28, line 4). The fragment comprising 1-73 of AtHD2A corresponds to amino acids 1-72 of AtHD2B

(nucleotides 61-276 of SEQ ID NO:7), and the fragment comprising amino acids 137-163 of AtHD2A corresponds to amino acids 154-198 of AtHD2B (nucleotides 522-655 of SEQ ID NO:7).

Therefore, Applicants submit that one of skill in the art would easily be able to determine the objected to sequences defined in claims 1 and 9.

However, in order to expedite prosecution of this application, Applicants have amended claims 1 and 9 to cancel, without prejudice, reference to nucleotides 49-267 and 457-534 of SEQ ID NO: 5, 61-855 of SEQ ID NO: 7, 61-655 of SEQ ID NO: 7, 61-276 of SEQ ID NO: 7, and 522-655 of SEQ ID NO: 7, and reserve the right to claim these sequences in a Divisional application.

V. SEQ ID NO:7

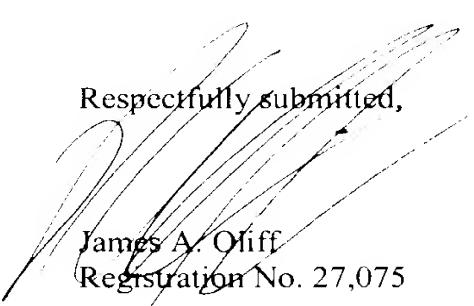
The Advisory Action requests that the differences between SEQ ID NO:7 as initially filed and as filed in the response of February 20, 2003 be pointed out. Attached please find a marked-up copy of SEQ ID NO:7 that indicates the changes made (nucleotides deleted at nucleotides 555, 577, 593, 627, 775-7, and --a-- inserted at position 665; numbering with reference to amended SEQ ID NO:7). These changes are supported in Figure 2B, as originally filed.

VI. CLOSING

In view of the foregoing amendments and remarks, Applicants submit that this application is in condition for allowance. Favorable reconsideration and prompt allowance of the claims are earnestly solicited.

Should the Examiner believe that anything further would be desirable in order to place this application in better condition for allowance, the Examiner is invited to contact Applicants' undersigned representative at the telephone number set forth below.

Respectfully submitted,


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Philip A. Caramanica, Jr.
Registration No. 51,528

JAO:PAC/jam

Attachments:

Appendix
Marked-Up Copy of SEQ ID NO:7

Date: April 21, 2003

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Telephone: (703) 836-6400

DEPOSIT ACCOUNT USE
AUTHORIZATION
Please grant any extension
necessary for entry;
Charge any fee due to our
Deposit Account No. 15-0461

APPENDIX

Changes to Claims:

Claims 50 and 54-56 are canceled.

The following is a marked-up version of the amended claims:

1. (Twice-Three Times Amended) A method of repressing ~~transcription~~ expression of a coding sequence of interest in a transgenic plant, comprising:
 - a) introducing into a plant:
 - i) a first chimeric nucleotide sequence comprising a first regulatory element in operative association with a ~~gene~~ said coding sequence of interest, and a controlling sequence; and
 - ii) a second chimeric nucleotide sequence comprising a second regulatory element in operative association with a nucleotide sequence encoding a histone deacetylase fused with a DNA binding protein, said DNA binding protein interacting with said controlling sequence, to produce said transgenic plant; and
 - b) growing said transgenic plant;
wherein said nucleotide sequence encoding a histone deacetylase is selected from the group consisting of:

AtRPD3A, a nucleotide sequence that hybridizes to *AtRPD3A* under a hybridization condition,

AtRPD3B, a nucleotide sequence that hybridizes to *AtRPD3B* under a hybridization condition,

AtHD2A, a nucleotide sequence that hybridizes to *AtHD2A* under a hybridization condition,

AtHD2B, a nucleotide sequence that hybridizes to *AtHD2B* under a hybridization condition,

nucleotides 1-1807 of SEQ ID NO:1, a nucleotide sequence that hybridizes to nucleotides 1-1807 of SEQ ID NO:1 under a hybridization condition,

nucleotides 142-1644 of SEQ ID NO:1, a nucleotide sequence that hybridizes to nucleotides 142-1644 of SEQ ID NO:1 under a hybridization condition,

nucleotides 1-1800 of SEQ ID NO:3, a nucleotide sequence that hybridizes to nucleotides 1-1800 of SEQ ID NO:3 under a hybridization condition,

nucleotides 121-1533 of SEQ ID NO:3, a nucleotide sequence that hybridizes to nucleotides 121-1533 of SEQ ID NO:3 under a hybridization condition,

nucleotides 1-939 of SEQ ID NO:5, a nucleotide sequence that hybridizes to nucleotides 1-939 of SEQ ID NO:5 under a hybridization condition,

nucleotides 49-783 of SEQ ID NO:5, a nucleotide sequence that hybridizes to nucleotides 49-783 of SEQ ID NO:5 under a hybridization condition,

nucleotides 49-681 of SEQ ID NO:5, a nucleotide sequence that hybridizes to nucleotides 49-681 of SEQ ID NO:5 under a hybridization condition,

nucleotides 49-534 of SEQ ID NO:5, a nucleotide sequence that hybridizes to nucleotides 49-534 of SEQ ID NO:5 under a hybridization condition,

~~nucleotides 49-267 and 457-534 of SEQ ID NO:5, a nucleotide sequence that hybridizes to nucleotides 49-267 and 457-534 of SEQ ID NO:5 under a hybridization condition;~~

nucleotides 1-1212 of SEQ ID NO:7, a nucleotide sequence that hybridizes to nucleotides 1-1212 of SEQ ID NO:7 under a hybridization condition, and

nucleotides 61-975 of SEQ ID NO:7, a nucleotide sequence that hybridizes to nucleotides 61-975 of SEQ ID NO:7 under a hybridization condition;

~~nucleotides 61-855 of SEQ ID NO:7, a nucleotide sequence that hybridizes to nucleotides 61-855 of SEQ ID NO:7 under a hybridization condition;~~

— nucleotides 61-655 of SEQ ID NO:7, a nucleotide sequence that hybridizes to nucleotides 61-655 of SEQ ID NO:7 under a hybridization condition, — nucleotides 61-276 and 522-655 of SEQ ID NO:7, and a nucleotide sequence that hybridizes to nucleotides 61-276 and 522-655 of SEQ ID NO:7 under a hybridization condition;

wherein each said hybridization condition is selected from the group consisting of:

hybridization in 4XSSC at 65°C, followed by washing in 0.1XSSC at 65°C for an hour,

hybridization in 50% formamide and 4XSSC at 42°C, followed by washing in 0.1XSSC at 65°C for an hour, and

hybridization in 0.5 M Na₂HPO₄ (pH 7.2), 7% SDS, and 1mM EDTA at 65°C, followed by washing for 15 minutes in 2 x SSC with 0.1% SDS at room temperature, then washing twice for 20 minutes in 0.1 x SSC, 0.1% SDS at 65°C; and

wherein said nucleotide sequence that hybridizes encodes a product that exhibits repression of gene expression activity.

9. (Twice Three Times Amended) An isolated nucleotide sequence, selected from the group consisting of:

- i) — SEQ ID NO:3, SEQ ID NO:5, or SEQ ID NO:7,
- ii) — a nucleotide sequence that hybridizes to SEQ ID NO:3, SEQ ID NO:5, or SEQ ID NO:7,
- iii) — a nucleotide sequence that hybridizes to nucleotides 1-1800 of SEQ ID NO:3 under a hybridization condition,
- iv) — nucleotides 121-1533 of SEQ ID NO:3,

v) — a nucleotide sequence that hybridizes to nucleotides 121-1533 of SEQ ID NO:3 under a hybridization condition,

vi) — nucleotides 1-939 of SEQ ID NO:5,

vii) — a nucleotide sequence that hybridizes to nucleotides 1-939 of SEQ ID NO:5 under a hybridization condition,

viii) — nucleotides 49-783 of SEQ ID NO:5,

ix) — a nucleotide sequence that hybridizes to nucleotides 49-783 of SEQ ID NO:5 under a hybridization condition,

x) — nucleotides 49-681 of SEQ ID NO:5,

xi) — a nucleotide sequence that hybridizes to nucleotides 49-681 of SEQ ID NO:5 under a hybridization condition,

xii) — nucleotides 49-534 of SEQ ID NO:5,

xiii) — a nucleotide sequence that hybridizes to nucleotides 49-534 of SEQ ID NO:5 under a hybridization condition,

~~xiv) — nucleotides 49-267 and 457-534 of SEQ ID NO:5,~~

~~xv) — a nucleotide sequence that hybridizes to nucleotides 49-267 and 457-534 of SEQ ID NO:5 under a hybridization condition;~~

xvi) — nucleotides 1-1212 of SEQ ID NO:7,

xvii) — a nucleotide sequence that hybridizes to nucleotides 1-1212 of SEQ ID NO:7 under a hybridization condition,

xviii) — nucleotides 61-975 of SEQ ID NO:7, and

xix) — a nucleotide sequence that hybridizes to nucleotides 61-975 of SEQ ID NO:7 under a hybridization condition;

~~xx) — nucleotides 61-855 of SEQ ID NO:7,~~

xxi) a nucleotide sequence that hybridizes to nucleotides 61-855 of SEQ ID NO:7 under a hybridization condition;

xxii) nucleotides 61-655 of SEQ ID NO:7;

xxiii) a nucleotide sequence that hybridizes to nucleotides 61-655 of SEQ ID NO:7 under a hybridization condition;

xxiv) nucleotides 61-276 and 522-655 of SEQ ID NO:7, and

xxv) a nucleotide sequence that hybridizes to nucleotides 61-276 and 522-655 of SEQ ID NO:7 under a hybridization condition;

wherein each said hybridization condition is selected from the group consisting of:

hybridization in 4XSSC at 65°C, followed by washing in 0.1XSSC at 65°C for an hour,

hybridization in 50% formamide and 4XSSC at 42°C, followed by washing in 0.1XSSC at 65°C for an hour, and

hybridization in 0.5 M Na₂HPO₄ (pH 7.2), 7% SDS, and 1 mM EDTA at 65°C, followed by washing for 15 minutes in 2 x SSC with 0.1% SDS at room temperature, then twice washing for 20 minutes in 0.1 x SSC, 0.1% SDS at 65°C; and

wherein said nucleotide sequence that hybridizes encodes a product that exhibits repression of gene expression activity.

APR 7 1 2003

<210> 7

<211> 1212

<212> DNA

<213> Arabidopsis thaliana

<400> 7

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gacagccttg tccacatttc tcaggcttca cttgactgca cagtgaaatc tggagaatct 180
gtggtttga gtgtgactgt tgggtgggct aaacttgtta ttggaacact ttcacaagac 240
aagttccctc agattagctt tgatttggtt tttgataaaag agtttgagct ttcacacacgc 300
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gatgacttagt atgatgacga ggaggaaggat tctgaggatg aagaagagga ggagactcct 660
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tctggaaaga aggcaaaacc agcagcagca ccagcttcta ctcctcagaa gacaggagagaag 780
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